



# Preparation and Antioxidant Activities of New Di-Amino-Schiff Base Derivatives of Chitosan

Zhimei Zhong<sup>\*</sup>, Guangnan Zhu, Zhihong Zhao, Haijie Li, Zhenting Zhang

College of Sciences, Inner Mongolia Agricultural University, Huhhot, China

## Email address:

zhimeihappy@126.com (Zhimei Zhong)

<sup>\*</sup>Corresponding author

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**Abstract:** The damage caused by reactive oxygen radicals to the body is huge, and it can lead to aging of the human body and cause the occurrence of many diseases. Therefore, finding new antioxidant substance is urgent. In order to obtain new non-toxic antioxidant reagent, 3 kinds of di-amino-Schiff base derivatives of chitosan were prepared by grafting urea molecule onto chitosan chain using chitosan as raw material in this paper. The reaction conditions such as different reaction time, temperature, solvent and the molar ratio of reaction materials were discussed, and the structures of the derivatives were characterized by FT-IR and <sup>13</sup>C NMR spectroscopy and elemental analysis. The antioxidant activities of chitosan and its derivatives were tested, including the scavenging ability of superoxide anions, hydroxyl radicals and DPPH radicals. The experimental results show that the derivatives and chitosan all have good antioxidant activities, of which the removal rate of HCS for ·OH is 78.2%; and the clearance of DSABHCS at concentration of 500 µg·mL<sup>-1</sup> toward O<sub>2</sub><sup>-</sup> is 97.8%; the clearance of LCS to DPPH is 94.3% at concentration of 600 µg·mL<sup>-1</sup>. These results laid the foundation for the development of chitosan and its derivatives using as new antioxidant reagents.

**Keywords:** Chitosan, Urea, Derivatives, Preparation, Antioxidant Activity

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## 1. Introduction

There has been increasing interest in finding natural antioxidants in recent years. Studies have shown that the damage caused by reactive oxygen radicals to the body is huge, and the presence of reactive oxygen species can lead to aging of the human body, and can also cause lesions in human organs such as lungs, hearts, liver, and skin. It even causes the occurrence of many diseases such as atherosclerosis, diabetes, and tumors [1-5]. As a natural renewable resource, chitosan has some good properties such as biocompatibility, biodegradability, non-toxicity, antibacterial [6-8] and antioxidant activity [9-10], which has attracted much scientific interests in biotechnology, cosmetics, agriculture, food science, and textiles etc. The antioxidant activities of chitosan and its derivatives has attracted the most attention. Xing et al had found that all kinds of sulfated chitosans possessed antioxidant activities and free radical scavenging activities [11]. Fang Luan et al.

successfully synthesized the water-soluble chitosan derivatives propane sulfonated chitosan and dipropene sulfonated chitosan, and evaluated the antioxidant activities of them. The results found that the best scavenging activity against superoxide-radical and DPPH-radical were 94.1% and 100% at 1.6 mg·mL<sup>-1</sup>, respectively [12].

The mechanism of scavenging activity of chitosan derivatives against free radicals was proposed by Xie et al [13]. They suggested ·OH can react with the free -NH<sub>2</sub> in chitosan chain to form stable macromolecules radicals. Besides, The -NH<sub>2</sub> can form -NH<sub>3</sub><sup>+</sup> by absorbing H<sup>+</sup> from the solution, and then they react with ·OH through addition reactions. Based on the above theories, we prepared 3 kind of new di-amino-Schiff base derivatives of chitosan with more -NH<sub>2</sub> groups to improve the antioxidant of chitosan.

## 2. Materials and Methods

### 2.1. Materials

Chitosan (CS) is a commercial material supplied by Qingdao Yunzhou Biochemical Corp. (China). They have deacetylation of 90%, average molecular weight (MW) 69,200 Da (HCS), 6200 Da (MCS) and 3100 Da (LCS). Nitro blue tetrazolium (NBT), phenazine mothosulfate (PMS), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), nicotinamide adenine dinucleotidereduced (NADH), ethylene diamine tetra-acetic acid (EDTA), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric chloride were purchased from Sigma Chemicals Co. All other chemicals and reagents, unless otherwise specified, were not purified, dried or pretreated.

### 2.2. Analytical Methods

Fourier transform infrared (FTIR) spectra of all of the compounds were measured in the  $4000\text{--}400\text{ cm}^{-1}$  regions using a MAGNA-IR5600 FT-IR spectrometer. The elemental analyses (C, H, N) were performed on a EA3000 elemental analyzer.  $^{13}\text{C}$ NMR spectra were recorded on a Jam-Ecp600 (600 MHz) NMR spectrometer in DAc solvent. The average viscometric molecular weight of chitosan and all of the

derivatives was estimated from the intrinsic viscosity determined in the solvent  $0.1\text{ mol}\cdot\text{L}^{-1}\text{ CH}_3\text{COOH}/0.2\text{ mol}\cdot\text{L}^{-1}\text{ NaCl}$  using the Mark-Houwink parameter  $\alpha=0.96$ ,  $K_\eta=1.424$  at  $25^\circ\text{C}$  when the intrinsic viscosity is expressed in  $\text{mL}\cdot\text{g}^{-1}$ .

### 2.3. General Procedure for the Synthesis of $C_2$ -di-amino-Schiff base-chitosan (DASBCS)

2g (0.012 mol) chitosan with different molecule weight was dissolved in a proper volume of 1% acetic acid (HAc) under nitrogen atmosphere. When the temperature was proper, HAc solution contained urea was added to the system. After stirring 10 minutes, potassium persulfate and sodium bisulfite were added to the system, and stirring for a few hours. The reaction mixture was concentrated to 50 mL and poured into a beaker containing 300 mL ethanol, giving a white precipitate. After placed at  $4^\circ\text{C}$  for 10 hours, The mixture of products was filtered through a Bucher funnel under reduced pressure. The precipitate was rinsed with ethanol and distilled water. The solution was dialyzed against distilled water for 48 h using a 2000 Da MW cut-off dialysis membrane. The product was then concentrated and lyophilized to give the urea derivatives of chitosan. Synthesis of the object chemicals is via the procedure as outlined in Figure 1.

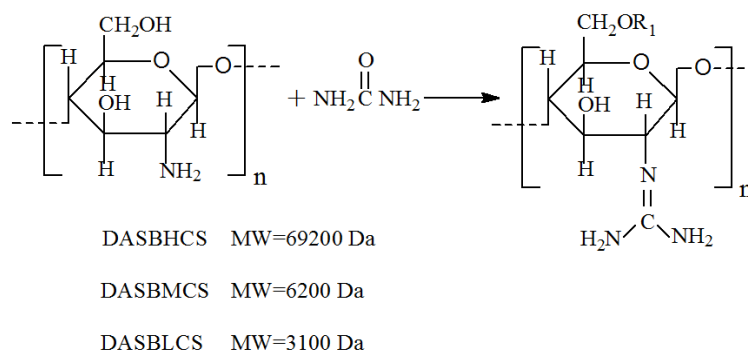


Figure 1. Synthesis pathway of  $C_2$ -di-amino-Schiff base-chitosan.

### 2.4. Antioxidant Activity

#### 2.4.1. Hydroxyl Radical Assay

The reaction mixture, containing all kinds of the prepared compounds ( $0.04\text{--}0.75\text{ mg}\cdot\text{mL}^{-1}$ ) was incubated with deoxyribose ( $3.75\text{ mM}$ ),  $\text{H}_2\text{O}_2$  ( $1\text{ mM}$ ),  $\text{FeCl}_3$  ( $100\text{ }\mu\text{M}$ ), EDTA ( $100\text{ }\mu\text{M}$ ) and ascorbic acid ( $100\text{ }\mu\text{M}$ ) in potassium phosphate buffer ( $20\text{ mM}$ ,  $\text{pH}7.4$ ) for 30 min at  $37^\circ\text{C}$ . [14] The reaction was terminated by adding 1 mL TBA ( $1\%$   $W/V$ ) and 1mL TCA ( $2\%$   $W/V$ ), and then heating the tubes in a boiling water bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 535 nm against blank. Decreased absorbance of the reaction mixture indicated oxidation of deoxyribose. The scavenging ability was calculated as follows:

$$E\% = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \times 100$$

#### 2.4.2. Superoxide-radical Scavenging Assay

The superoxide radicals scavenging ability the compounds were assessed by the method of Nishikimi et al. [15] The reaction mixture containing sample ( $0.04\text{--}0.45\text{ mg}\cdot\text{mL}^{-1}$ ), PMS ( $30\text{ }\mu\text{M}$ ), NADH ( $338\text{ }\mu\text{M}$ ), and NBT ( $72\text{ }\mu\text{M}$ ) in phosphate buffer ( $0.1\text{ mol}\cdot\text{L}^{-1}$ ,  $\text{pH}7.4$ ) was incubated at room temperature for 5 min and the absorbance was measured at 560 nm against a blank. The capability of scavenging superoxide radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left( 1 - \frac{A_{\text{sample}560\text{nm}}}{A_{\text{control}560\text{nm}}} \right) \times 100$$

#### 2.4.3. DPPH Free Radical's Scavenging Assay

DPPH free radical's scavenging assay was performed as the method of Curcio et al [16] with some modification. DPPH was dissolved with anhydrous ethanol and the

concentration was  $180 \mu\text{mol}\cdot\text{L}^{-1}$ . Dissolved every sample with anhydrous ethanol. Chitosan sample (1 mL) was added into the 0.2 mM radical solution. And the final concentrations of every sample was  $25 \mu\text{g}\cdot\text{mL}^{-1}$ ,  $50 \mu\text{g}\cdot\text{mL}^{-1}$ ,  $75 \mu\text{g}\cdot\text{mL}^{-1}$ ,  $100 \mu\text{g}\cdot\text{mL}^{-1}$  and  $200 \mu\text{g}\cdot\text{mL}^{-1}$ . The mixture was shaken and incubated for 30 min at room temperature. The absorbance was then measured at 517 nm using a spectrophotometer. The DPPH radical-scavenging capacity was estimated based on the difference in absorbance with or without samples and expressed as a percentage of DPPH scavenging at 4°C. The control of this assay was made up of secondary distilled water and DPPH solution. Replace DPPH solution of the sample groups with anhydrous ethanol to get the blank samples. The clearance could be got from the bellow equation:

$$E(\%) = [1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100$$

Where  $A_{\text{sample}}$ ,  $A_{\text{blank}}$  and  $A_{\text{control}}$  were the absorbance of sample solutions, blank solution and the control solution respectively. Each experiment was performed three times, and the data were averaged.

## 2.5. Statistical Analysis

All data are expressed as means  $\pm$  SD. Data were analyzed by an analysis of variance ( $P < 0.05$ ) and the means separated by Duncan's multiple range tests. The results were processed by computer programs: Excel and Statistic software (2013).

# 3. Results and Discussion

## 3.1. Preparation of DASBCS

### 3.1.1. Effect of Reaction Time, Temperature, Dosage of Crosslinking Agent and the Molar Ratio of Reaction Materials on DASBCS

Figures 2-3 show the results of 3 kinds of DASBCS yield under different dosage of crosslinking agent ( $\text{K}_2\text{S}_2\text{O}_8$  and  $\text{NaHSO}_3$ ). When the dosage of crosslinking agent changed, yield changed at the same time. Yield increased with the increase of the concentration of  $\text{K}_2\text{S}_2\text{O}_8$  (below  $3.00 \text{ mmol}\cdot\text{L}^{-1}$ ) and  $\text{NaHSO}_3$  (below  $2.50 \text{ mmol}\cdot\text{L}^{-1}$ ), but the yield decreased slowly after the concentration beyond the above listed concentration. As a result, the proper dosage of crosslinking agent is  $3.00 \text{ mmol}\cdot\text{L}^{-1} \text{K}_2\text{S}_2\text{O}_8$  and  $2.50 \text{ mmol}\cdot\text{L}^{-1} \text{NaHSO}_3$ .

As shown in Figure 4, yield changed with the increase of molar ratio, and the maximum yield (72.35%) appeared at the molar ratio is 1:2. As a result, 1:2 is the proper molar ratio.

Figures 5-6 depict yield changing with the time and temperature. An increase in reaction temperature caused yield increased. But yield decreased obviously when the temperature reached  $75^\circ\text{C}$ . This result indicates that the high reaction temperature maybe cause to the degradation of chitosan. Therefore, the following experiments were carried at  $70^\circ\text{C}$ . The yield changed with prolonging reaction time. Yield did not increased after 5 hours, so 5 hours is the proper time. All these results showed that the reaction depends on both reaction time and temperature.

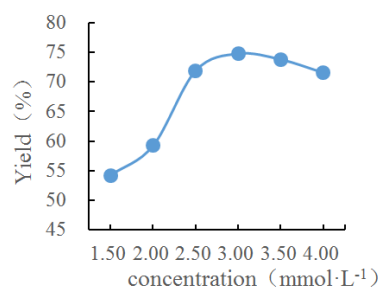


Figure 2. The effect of  $\text{K}_2\text{S}_2\text{O}_4$  concentration on yield.

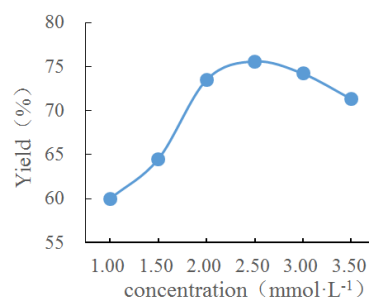


Figure 3. The effect of  $\text{NaHSO}_3$  concentration on yield.

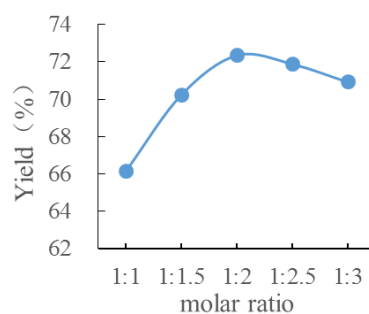


Figure 4. The effect of molar ratio of chitosan and urea on yield.

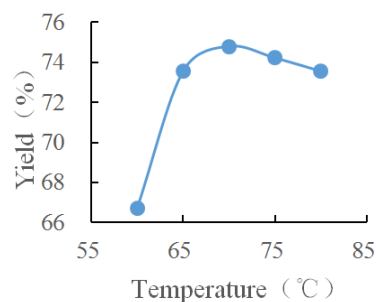


Figure 5. The effect of reaction temperature on yield.

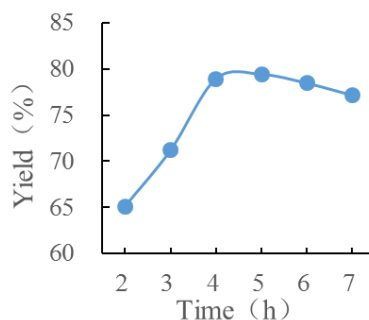


Figure 6. The effect of reaction time on yield.

### 3.1.2. Structure and Physicochemical Characteristics of the Compounds

Figure 7 presents the comparison of transmission FT-IR spectra data for UREA, DASBHCS, DASBMCS and DASBLCS with original CS. As for the FT-IR spectra of CS with DASBCS are concerned, firstly, obvious translocation at  $3500\text{--}3200\text{ cm}^{-1}$  due to the O-H and N-H group stretching vibration were observed, at the same time, the breadth of the peak broaden comparing with the spectra of CS, because the more  $\text{-NH}_2$  appeared. In addition, the characteristic absorbance of  $\text{-NH}_2$  at  $2700\text{ cm}^{-1}$  can be observed. Secondly, new peaks at about  $1700\text{ cm}^{-1}$  appeared at the spectra of DASBHCS, DASBMCS and DASBLCS is the characteristic absorbance of carbonyl-group. Above-mentioned results demonstrated that DASBCS were formed successfully.

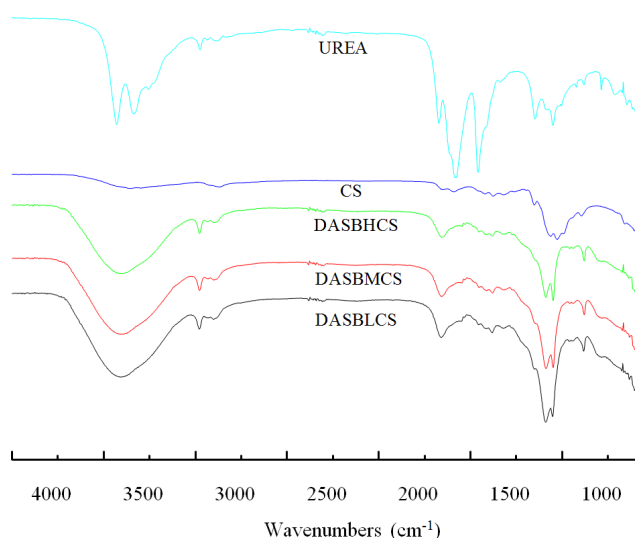


Figure 7. FT-IR spectra of urea, chitosan and its derivatives.

The results of elemental analyses, yield and substituting degree of DASBCS are listed in table 1. The elemental analyses indicate that the  $\text{C}_2$ -urea-substitution of DASBHCS, DASBMCS and DASBLCS is 39.53%, 37.97 and 37.17 respectively. Yield is changed from 70.5 to 76.2 for all of the 3 derivatives of chitosan.

Table 1. Elemental analyses, yield, and substituting degree of DASBCS.

compound	Yield (%)	Elemental analyses (%)				substituting degree (%)
		C	H	O	N	
CS	-	45.16	7.18	38.91	8.75	-
DASBHCS	76.18	40.31	5.91	32.05	21.73	39.53
DASBMCS	72.53	41.17	5.39	31.64	21.80	37.97
DASBLCS	70.57	42.39	5.02	30.02	22.57	37.17

Figure 8 depicts the  $^{13}\text{C}$  NMR spectrum of CS, DASBHCS, DASBMCS and DASBLCS. In the spectra of CS, the peaks at  $\delta=97.58, 55.71, 70.07, 76.22, 74.69$  and  $59.83\text{ ppm}$  are attributed to the C-1, C-2, C-3, C-4, C-5 and C-6 in chitosan molecule, respectively. The peaks at  $\delta=178.59$  is the characteristic absorbance of  $\text{C=O}$  in acetyl group. The peaks at  $\delta=20.68\text{--}22.00\text{ ppm}$  are the absorbance of  $\text{-CH}_3$  and  $\text{-CH}_2$  groups.

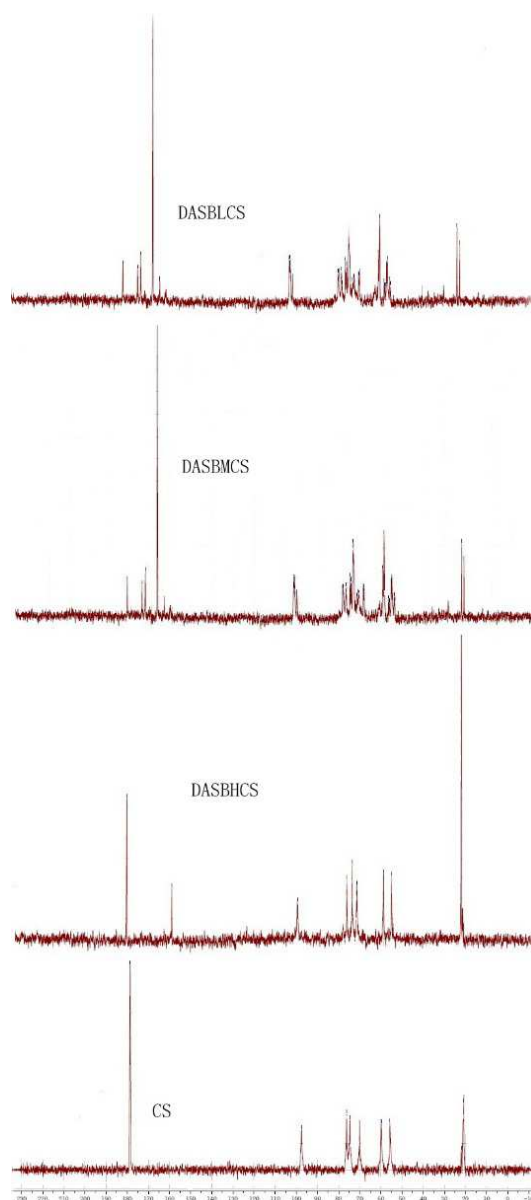


Figure 8.  $^{13}\text{C}$  NMR spectrum of chitosan and its derivatives.

As for the spectra of DASBHCS is concerned, the peaks appearing at  $181.40\text{ ppm}$  is the peak of  $\text{C=O}$ , and the peaks at about  $\delta=23\text{ ppm}$  are attributed to  $\text{-CH}_3$  and  $\text{-CH}_2$  groups. The peaks at  $\delta=100.76, 56.23, 72.63, 77.35, 74.78$  and  $60.01\text{ ppm}$  were attributed to the C-1, C-2, C-3, C-4, C-5 and C-6 in chitosan molecular, respectively. The above results indicated that DASBHCS was obtained successfully.

The peaks of spectra of DASBMCS and DASBLCS are listed as follows. DASBMCS,  $\delta=180.92\text{ ppm}$  ( $\text{C=O}$  in chitosan chain),  $\delta=23\text{ ppm}$  ( $\text{-CH}_3$  and  $\text{-CH}_2$ ),  $\delta=100.06$  (C1),  $\delta=56.51\text{ ppm}$  (C2),  $\delta=60.01\text{ ppm}$  (C6),  $\delta=72.53\text{ ppm}$  (C3),  $\delta=74.65\text{ ppm}$  (C5),  $\delta=77.17\text{ ppm}$  (C4),  $\delta=163.41\text{ ppm}$  ( $\text{C=O}$  in urea group). DASBLCS,  $\delta=181.43\text{ ppm}$  ( $\text{C=O}$  in chitosan chain),  $\delta=23\text{ ppm}$  ( $\text{-CH}_3$  and  $\text{-CH}_2$ ),  $\delta=102.48\text{ ppm}$  (C1),  $\delta=56.25\text{ ppm}$  (C2),  $\delta=60.55\text{ ppm}$  (C6),  $\delta=72.75\text{ ppm}$  (C3),  $\delta=74.57\text{ ppm}$  (C5),  $\delta=77.72\text{ ppm}$  (C4),  $\delta=163.93\text{ ppm}$  ( $\text{C=O}$  in urea group).



### 3.2. Antioxidant Activities

#### 3.2.1. Hydroxyl Radical Scavenging Activity of CS and DASBCS

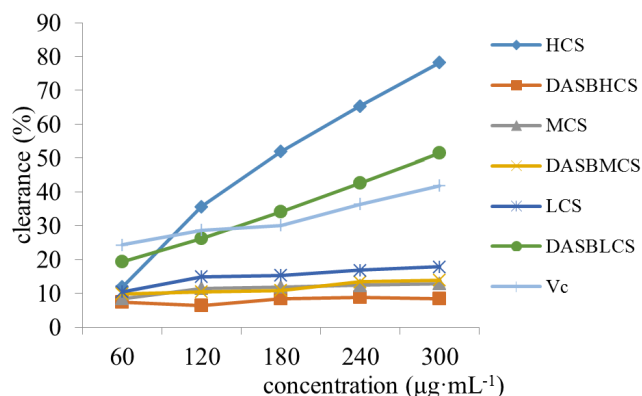


Figure 9. Scavenging curves of urea derivatives of chitosan towards hydroxyl radical.

Scavenging results of urea derivatives of chitosan towards hydroxyl radical are listed in figure 9. As shown in figure 8, the scavenging rate increased with increasing concentration. HCS has the most obvious scavenging activity, and the highest clearance is 78.2% at the concentration of 300  $\mu\text{g}\cdot\text{mL}^{-1}$ .  $\text{IC}_{50}$  of HCS is 178  $\mu\text{g}\cdot\text{mL}^{-1}$ . The antioxidant activity of DASBHCS is low, and the clearance is between 6% and 8% in the range of tested concentration, which indicates that the introduction of more amino groups does not improve the scavenging ability of HCS to hydroxyl groups. Both clearance of MCS and DASBMCS were low, ranging from 9% to 13%. The activity of DASBLCS was higher than that of LCS. The clearance of LCS and DASBLCS were 17.8% and 51.5% respectively at 300  $\mu\text{g}\cdot\text{mL}^{-1}$ , and the antioxidant activity of DASBLCS increased significantly with the increase of concentration. Analyzing the influence of molecular weight on scavenging ability of CS and its derivatives toward hydroxyl radicals, it was found that molecular weight of CS had no obvious effect on activity. For its derivatives, with the increase of molecular weight, the scavenging rate of derivatives increased significantly. Furthermore, the clearance of HCS and DASBLCS on hydroxyl radicals were significantly higher than those of Vc (Its maximum scavenging rate was 41.8%) when the concentration of HCS and DASBLCS was higher than 180  $\mu\text{g}\cdot\text{mL}^{-1}$ . The results showed that these two substances have potential to be developed and applied as new antioxidants.

The above results showed that the introduction of more amino groups significantly enhanced the antioxidant activity of chitosan for LCS, suggesting that the scavenging effect of LCS on hydroxyl radicals was mainly due to the grafted urea group contained more active  $-\text{NH}_2$  group, which can react with hydroxyl radical to form stable macromolecular radicals. For the poor water solubility MCS, the introduction of  $-\text{NH}_2$  group had little effect on the scavenging activity. At the same time, the antioxidant capacity of water-insoluble chitosan (HCS) decreases with the introduction of excessive  $-\text{NH}_2$ , which

indicates that the scavenging capacity of HCS to hydroxyl radicals is not the action of  $-\text{NH}_2$ , but it is related to the effect of  $-\text{OH}$  in chitosan molecules. In addition, chitosan urea derivatives with low molecular weight have stronger scavenging efficiency.

This result may be caused by intramolecular and intermolecular hydrogen bonds of low molecular weight DASBLCS were weaker than that of high molecular ones, for LCS and DASBLCS have less-compact structure, and the effect of the intramolecular hydrogen bonds is weak, so hydroxyl radical can react with free hydroxyl and amino group in chitosan chain.

#### 3.2.2. Scavenging Activity of Superoxide Radical by CS and DASBCS

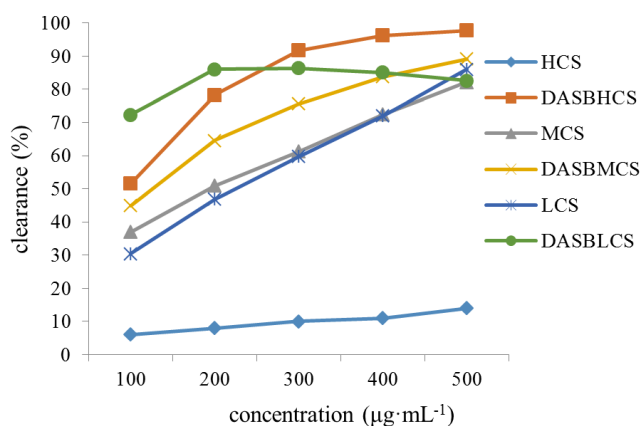


Figure 10. Scavenging activity of CS and DASBCS towards superoxide anion.

The results of Figure 10 show that the scavenging rate of all compounds on  $\text{O}_2^-$  increases with the increase of concentration. The clearance of HCS is low (the value is between 6-14%) and does not change obviously with the concentration. Others have strong antioxidant effect on  $\text{O}_2^-$  with the maximum clearance value above 80%. The  $\text{IC}_{50}$  of DASBHCS and DASBLCS is below 100  $\mu\text{g}\cdot\text{mL}^{-1}$ . The clearance of DASBHCS at 500  $\mu\text{g}\cdot\text{mL}^{-1}$  is 97.8%. The influence of molecular weight on clearance was analyzed, and the results showed that the lower the molecular weight was, the greater the scavenging effect of CS on  $\text{O}_2^-$  was, and the clearance of LCS was 86.0%. However, for DASBCS, the higher the molecular weight was, the higher the clearance was. Comparing the antioxidant activity of chitosan and its derivatives, it was found that the activity of all urea derivatives was much higher than that of chitosan, which indicated that the higher scavenging effect was caused by more amount of  $-\text{NH}_2$  introduced onto chitosan chain. The scavenging effect of ascorbic acid (Vc) on  $\text{O}_2^-$  was tested in the experiment, but the results showed that the scavenging rate of Vc was low, and its value was less than 5%. (Which was not shown in figure 10).

All of the above results indicate that  $-\text{OH}$  and  $-\text{NH}_2$  in chitosan molecule can react with  $\text{O}_2^-$  and achieve the purpose of scavenging  $\text{O}_2^-$  in chitosan molecule. In addition, the

higher the molecular weight of chitosan, the worse the scavenging effect of  $O_2^-$ , which is consistent with the previous analysis results [17]. The mechanism of these results is that the inner structure of chitosan was severely disrupted by the introduction of grafted urea after modification. The ability to form hydrogen bond declines sharply, and the hydroxyl and amino groups are activated, and the numbers of amino groups is increased, so this is helpful to the reaction with superoxide anion. In conclusion, these results suggested that the antioxidant activity of urea derivatives of chitosan was related to its ability to scavenge superoxide radical.

### 3.2.3. Scavenging Ability on DPPH

As shown in Figure 11, the scavenging effect of all the tested compounds on DPPH radicals increased with the decrease of molecular weight and the increase of concentration. The antioxidant activity of MCS and LCS was stronger than that of DASBMCS and DASBLCS, while that of DASBHCS was stronger than that of HCS, but both values were lower (both below 10%). The results showed that the introduction of multiple amino groups in urea onto chitosan chain did not improve the scavenging capacity of chitosan for DPPH. In addition, the clearance of LCS reached 94.3% at the concentration of  $600\mu\text{g}\cdot\text{mL}^{-1}$ , which laid a foundation for using LCS as DPPH scavenger.

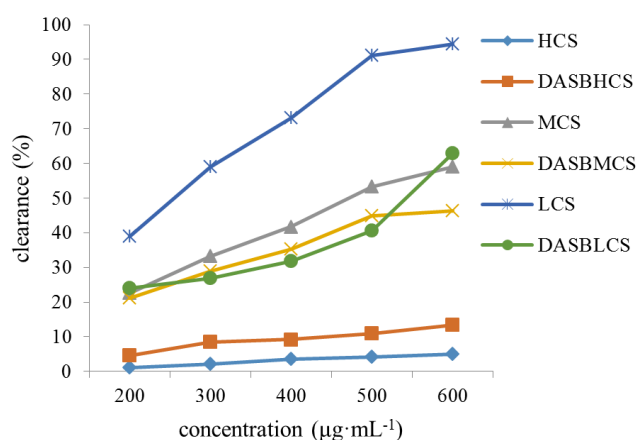


Figure 11. Scavenging effect of CS and DASBCS on DPPH radicals.

The above experimental results are consistent with the structure and antioxidant mechanism of DPPH radicals. DPPH is a very stable free radical centered on nitrogen atom. Its stability mainly comes from the resonance stabilization of benzene ring in its molecule and the large space obstacle, which makes the paired electrons of nitrogen atom in its molecule unable to play their due role in pairing electrons.

Antioxidant reagent reacts with DPPH mainly through the N atom in DPPH radical. If the free radical scavenger reacts with the single electron in N atom, it will scavenge the DPPH radical. Because of its good water solubility, low molecular weight and small steric hindrance, LCS has more possibility of contacting N atom in DPPH molecule, thus destroying the

stability of DPPH and reacting with DPPH to clear it. However, the existence of hydrogen bond in HCS long chain make it difficult to react with the N atom in DPPH, resulting in the low activity of HCS and DASBHCS.

The scavenging effect of ascorbic acid on DPPH was tested in the experiment, and the results showed that the scavenging effect of Vc on DPPH was very strong (the scavenging rate was above 90%). The mechanism is that ascorbic acid has strong reducibility, which can reduce  $N=N$  in DPPH molecule and decompose it into conjugated products to achieve the purpose of scavenging [18].

## 4. Conclusion

In this paper, 3 new urea derivatives of chitosan were prepared according to the optimal synthesis conditions: 1% HAc as solvent, reaction were carried at  $70^\circ\text{C}$ , the proper reaction time was 5 hours; The molar ratio of urea and chitosan was 2:1, the proper concentration of crosslinking agent is  $3.00\text{ mmol}\cdot\text{L}^{-1}$   $\text{K}_2\text{S}_2\text{O}_8$  and  $2.50\text{ mmol}\cdot\text{L}^{-1}$   $\text{NaHSO}_3$ . The structure of the new derivatives were characterized by FTIR,  $^{13}\text{C}$  NMR and elemental analysis, and the urea substituting degree of the new urea derivatives were above 37%. Furthermore, as expected, we obtained several satisfying results of the antioxidant action of the compounds. The results showed that DASBHCS, DASBMCS and DASBLCS had stronger scavenging effect on superoxide anions than Vc and chitosan, with scavenging rates of 97.8%, 89.2% and 82.6%, respectively. DASBLCS had stronger scavenging effect on hydroxyl radicals than Vc. All of the results indicated that the urea group polymerized on chitosan can increase its antioxidant activity obviously. The mechanism of the new prepared compounds toward  $\cdot\text{OH}$ ,  $O_2^-$  and DPPH was studied at the same time. These assays had important applications for the new derivatives of chitosan using in pharmaceutical and food industries, and they had the potential ability to develop new antioxidant reagents.

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